Mechanisms of Antibiotic Resistance Determined by Resistance-Transfer Factors

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ABSTRACT

Unowsky, Joel (Northwestern University Medical School, Chicago, Ill.), and Martin Rachmeler. Mechanisms of antibiotic resistance determined by resistance-transfer factors. J. Bacteriol. 92:358-365. 1966.—This study was concerned with the mechanism of expression of drug resistance carried by resistance-transfer (R) factors of two types: fi− (negative fertility inhibition) and fi+ (positive fertility inhibition). The levels of drug resistance determined by R factors used in this study were similar to those reported by other investigators. A new finding was that Escherichia coli carrying the fi− episome was resistant to 150 to 200 μg/ml of streptomycin. The growth kinetics of R factor-containing cells were similar in the presence or absence of streptomycin, chloramphenicol, and tetracycline, but a period of adaptation was necessary before cells began exponential growth in the presence of tetracycline. By use of radioactive antibiotics, it was shown that cells containing the fi− episome were impermeable to tetracycline and streptomycin, whereas cells containing the fi+ episome were impermeable only to chloramphenicol. Cell-free extracts from fi+ and fi− cells were sensitive to the antibiotics tested in the polyuridylic acid-stimulated incorporation of phenylalanine into protein.

Resistance-transfer factors (R factors) are episomes isolated from members of the Enterobacteriaceae. They carry genetic information to confer resistance to one or more of the drugs, streptomycin (SM), chloramphenicol (CM), tetracycline (TC), and sulfonamide (SU), as well as to penicillin, kanamycin, neomycin, and phleomycin (21, 23). Interest in these factors was first stimulated by their interference in the chemotherapy of bacillary dysentery in Japan (19); these episomes have since become of worldwide interest, appearing also in Germany, Israel (20), and England (3). Although the genetics of these episomes has been extensively analyzed (11, 15, 20), little is known about the mechanism of drug resistance. Several investigators (17, 18) examined the cell-free amino acid-incorporating systems from cells with and without R factors. CM, SM, and TC inhibited the incorporation of amino acids by cell-free extracts of both drug-resistant (R+) and drug-sensitive (R−) cells of Escherichia coli. From the above indirect evidence, impermeability seemed a likely mechanism by which a cell resisted these drugs. This method of postulating impermeability as a mechanism of drug resistance, based on the in vitro sensitivity of enzymes to a drug, is a widely used method (16).

During the course of our study of R factors, radioactive TC, CM, and SM became available. Several investigations have been carried out with these radioactive antibiotics, comparing the uptake in cells that are chromosomally resistant with uptake in sensitive ones. By use of this method, impermeability was postulated as a mechanism of resistance to TC (6), CM (19), and SM (9). In our study, we examined the permeability of cells containing R factors by use of radioactive drugs.

The most commonly isolated type of R factor inhibits F-mediated chromosomal transfer in the Enterobacteriaceae. It carries factors for resistance to the four drugs, TC, SM, SU, and CM, and is designated fi+ (8). Recently, a second type of R factor, which can actually mediate chromosomal transfer, has been recognized (22). This second type, designated fi−, lacks CM resistance (chl-s) and is also distinguished by several other
genes. In our studies, we compared cells carrying an epism of each type with sensitive cells.

**Materials and Methods**

**Bacterial strains.** E. coli strains CSH-2 (met) and W-677 (pre-T6-r str-r thr-leu- B; mar xyl- mut-gal- lac-) were kindly supplied by T. Watanabe. A. B. Pardee supplied two substrains of E. coli K-12: 180 (his cys) and 3000 (B+). Shigella flexneri 2b (metry nie) was kindly supplied by R. Nakaya.

**R factors.** The strain CSH-2 contained either the R factor 222, f1 carrying the drug-resistance markers denoting resistance to SM, CM, SU, and TC (str-r chl-r sul-r tet-r), or the R factor N2 (f1 str-r sul-r tet-r). S. flexneri contained the R factor f1. In this paper we will consider the R factors as representatives of their class, f1 or f2, rather than by their individual designations, N2 and 222.

**R transfer.** The R factor f1 was passed from S. flexneri to E. coli 180 or 3000. The donor and recipient were mixed in Difco Penassay Broth and spread on minimal medium containing 0.2% glucose and supplemented with 20 μg/ml of histidine and cysteine and 25 μg/ml of SM for the passage to 180; the medium was supplemented with 20 μg/ml of vitamin B1 for the passage to 3000. These strains were then designated as 180 f1 and 3000 f1.

**Culture conditions.** Complete broth contained 1% tryptone and 0.5% yeast extract. Cultures were maintained on complete agar (complete broth plus 1.5% agar). E.M.B. Agar (Difco) was used to test for the fermentation of lactose. Antibiotic resistance was tested on complete agar supplemented with (per milliliter) 25 μg of TC, 25 μg of CM, or 10 to 1,000 μg of SM, or combinations. Mueller Hinton medium (Difco) containing 500 μg of sulfathiazole/ml was used to test for SM resistance.

**Levels of drug resistance.** The levels of drug resistance were determined in complete broth and in Difco nutrient broth. Dilutions of the various antibiotics were made in the above broths, which were inoculated with 106 cells of each strain tested. Resistance to a given level of antibiotic was determined by measurement of turbidity after 18 hr of growth at 37 C in a Klett-Summerson colorimeter with a no. 54 filter.

**Growth curves.** Complete broth or nutrient broth was inoculated with 2 × 105 cells per milliliter from an overnight culture and shaken at 37 C. Klett units or optical densities at 550 nm and viable cell counts on complete agar were determined from samples every 40 min. Growth curves were obtained in the absence and presence of 25 μg of TC, 25 μg of CM, or 5 μg of SM/ml.

**Uptake of SM.** H4-dihydrosreptomycin sulfate (specific activity, 8 μc/mg) was generously supplied by the Merck Sharp and Dohme Research Laboratories Division of Merck & Co., Inc. Cells with and without R factors were grown in nutrient broth to early log phase (6 × 109 to 1 × 1010 cells per milliliter). A mixture of 5 ml of these cells and 25 μg of radioactive SM was incubated in a shaking water bath at 37 C. 1-ml samples were withdrawn every 15 min and filtered through membrane filters (HA, 25 mm, 0.45-μ pore size; Millipore Filter Corp., Bedford, Mass.). The cells were washed on the filters once with 5 ml of nutrient broth, and the filter was then added to 10 ml of scintillation fluid (12) in a counting vial and counted in a Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). In some experiments, 25 μg/ml of CM or TC was added 5 min before SM, to study their effects on the uptake and lethal activity of SM.

**Uptake of TC.** H4-TC (specific activity, 1 mc/mg) was purchased from the New England Nuclear Corp., Boston, Mass. An overnight culture grown in nutrient broth was diluted 40:1 in nutrient broth and grown for an additional 2 hr, giving a Klett reading of 20 to 30. To 10 ml of this broth culture was added 106 counts/min of radioactive TC and 10 μg/ml of nonradioactive TC. After 0, 20, 40 and 60 min of incubation, 2 ml was withdrawn, chilled immediately in an ice bath, and centrifuged at approximately 1,800 × g for 10 min. Filtration could not be used because TC remained attached to the filter and could not be quantitated. The cells were washed twice with 2 ml of nonradioactive medium. The radioactivity was determined by counting 0.1 ml of the resuspended cells in 10 ml of scintillation fluid. For studies on the effects of preincubation, overnight cultures of resistant cells were diluted in fresh nutrient broth containing 10 μg of TC/ml and grown to a Klett reading of 30; 10-ml samples were withdrawn, washed twice with nutrient broth, and tested for TC uptake as above.

**Uptake of CM.** C14-CM (specific activity, 8.5 mc/mg) was obtained from Calbiochem, CM, like TC, could not be assayed by filtration because it remained attached to the filter. There was very little uptake observed under the conditions used, as was also noted by Vazquez (19); it was, therefore, necessary to concentrate log-phase cells 40-fold for these experiments. C14-CM (106 counts/min) plus 100 μg of nonradioactive CM was added to 10 ml of concentrated culture. After 0, 20, 40, and 60 min of incubation at 37 C, 2-ml samples were withdrawn and assayed as with TC.

**Incorporation of radioactive phenylalanine by cell-free extracts.** For the preparation of extracts, cells were grown with aeration at 37 C in 16-liter carboys containing minimal medium supplemented with 0.2% each of yeast extract, peptone, tryptone, and glucose. After incubation with 500 ml of an overnight culture, the cells were in mid-log phase within 4 to 5 hr. The cells were collected by continuous-flow centrifugation in a Sharples or Sorvall centrifuge, and were stored at −20 C in portions (12 to 14 g) wrapped in Parafilm. For the preparation of extracts, the cells were suspended to 2.5 times their own weight in tris(hydroxymethyl) aminomethane (Tris) buffer (2) and treated in a Branson sonic oscillator at full strength for 5 min. The extract was centrifuged at 30,000 × g for 10, 10, and 20 min in a Sorvall RC-2 centrifuge; the pellet was discarded after each centrifugation. The method of extract preparation and assay was that of Matthaei and Nirenberg (14). Dodecylribonuclease acid and messenger ribonucleic acid were removed from the S100 fraction by passage through a diethylaminoethyl cellulose column in the cold.
The reaction mixture contained (in micromoles per milliliter): Tris (pH 7.8), 100; magnesium acetate (4), 20; mercaptoethanol, 6; adenosine triphosphate, 1; and phosphoenolpyruvate (K salt), 5. In addition, the mixture contained 2 µg of phosphoenolpyruvate kinase, 0.05 µg of C14-phenylalanine, 0.03 µg each of guanosine triphosphate, cytosine triphosphate, and uridine triphosphate, 20 µg of polyuridylic acid, 1.0 mg of E. coli soluble ribonucleic acid (sRNA), 1 mg of ribosomal protein, and 1 mg of S100 protein, in a total volume of 1 ml. The mixture was incubated for 50 min at 37 C, and the reaction was stopped by the addition of 4 ml of cold 5% trichloroacetic acid. Protein precipitation was aided by the addition of 20 µg of bovine serum albumin/ml as a carrier (2). The hot trichloroacetic acid precipitate was dissolved in 2 ml of strong base, 10% KOH or NaOH, and 0.1 ml was added to 10 ml of scintillation fluid and counted. When SM was used to inhibit incorporation, it was added prior to the polyuridylic acid, and the reaction mixture was incubated at 37 C for 10 min. The tubes were then chilled, polyuridylic acid was added, and the tubes were incubated for an additional 50 min at 37 C. When TC and CM were used, preincubation was carried out for 10 min without the RNA in the mixture. After chilling, sRNA was added and the incubation was continued for 50 min. Protein was determined by the method of Lowry et al. (13).

RESULTS

Growth and its inhibition by antibiotics. The growth curves of drug-resistant and drug-sensitive strains of E. coli K-12 in the presence and absence of the antibiotics are shown in Fig. 1. In log phase, all had a generation time of approximately 40 min in complete or nutrient broth. Growth of the sensitive strains 180 and CSH-2 was completely inhibited by the three antibiotics.

The resistant strains, CSH-2-fi+, CSH-2-fi−, and 180-fi+ showed identical growth in the presence or absence of drugs to which they were resistant; however, these resistant strains required a lag period of 2 hr before exponential growth began in the presence of TC. All cells of the TC-resistant strains, taken at various stages of growth in TC, including the lag, were TC-resistant. This was shown by replica-plating the colonies from complete media to complete media containing TC and noting that all the colonies grew. Colony counts were also identical when tested on complete and complete plus TC media. These latter findings suggested that the phenomenon involved in the growth lag in the presence of TC was adaptation rather than selection or segregation of the epsiome-containing cells. That this phenomenon could occur in the presence of TC was made understandable by the observation that TC only partially inhibited protein synthesis in these cells, thereby apparently allowing production of required products.

Levels of antibiotic resistance. The drug sensitivity of R factor-containing and R factor-lacking cells is shown in Table 1. Although all cells resistant to CM and TC survived a concentration of 100 to 150 µg/ml, the level of resistance to SM varied with the source of the resistant locus. The str-r strain W-677 was resistant to over 1,000 µg of SM/ml, as was S. flexneri when it contained the epsiome fi+. However, strains of E. coli K-12 containing the same epsiome, CSH-2-F−fi+ and 180-fi+, were resistant to a much lower level of SM (10 to 25 µg/ml). When the fi+ epsiome was passed by conjugation from S. flexneri to E. coli 180, the resultant E. coli strain was resistant to the lower level of SM. Strain CSH-2-F−, containing fi−, was resistant to an intermediate level of SM (150 to 200 µg/ml).

Uptake of SM. SM-resistant cells were divided into two groups on the basis of uptake (Fig. 2). The first group, which included the str-r cells and those containing the fi− epsiome, were relatively impermeable to SM. The second group, comprised of the fi+ epsiome-containing cells,
TABLE 1. Levels of drug resistance expressed by various strains in complete broth

<table>
<thead>
<tr>
<th>Strain</th>
<th>Resistance level (μg/ml)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>SM</td>
</tr>
<tr>
<td>Escherichia coli K-12</td>
<td>1,000</td>
</tr>
<tr>
<td>W-677 (str- r)</td>
<td>1,000</td>
</tr>
<tr>
<td>E. coli K-12 CSH-2-F^-</td>
<td>10-25</td>
</tr>
<tr>
<td>N1 .fr^-</td>
<td>150-200</td>
</tr>
<tr>
<td>E. coli K-12 CSH-2-F^-</td>
<td>10-25</td>
</tr>
<tr>
<td>E. coli K-12 (180)</td>
<td>10-25</td>
</tr>
<tr>
<td>Shigella flexneri 2b-222</td>
<td>1,000</td>
</tr>
</tbody>
</table>

Fig. 2. Uptake of SM by str-s strains, CSH-2-F^- (●) and 15-51, (■) and str-r strains, CSH-2-F^-fi+ (○), CSH-2-F^-fi+ (△), W-677 (▲), and 15.49 (□).

Fig. 3. Uptake of CM by CM-resistant cells, CSH-2-F^-fi+ (○), and sensitive cells, CSH-2-F^- (●).

These results eliminated permeability as a mechanism of resistance to SM for those cells containing the fi+ episome.

Uptake of CM. Uptake of CM in resistant and sensitive cells was essentially completed after 20 min (Fig. 3). The resistant cells incorporated much smaller amounts than did the sensitive cells. The fi- episome does not determine CM resistance, and cells containing this episome took up CM as well as did the sensitive cells. Hurwitz and Rosano (10) have shown that, in the presence of CM, the uptake and lethal effects of SM in E. coli were blocked. Since there was a permeability barrier to CM in our resistant cells, CM should have no effect on the uptake of SM. In fact, this was shown to be the case (Fig. 4). The addition of 5 μg of SM/ml to SM-sensitive cells resulted in a 90% loss of viability in 20 min and a 99% loss within 1 hr of incubation. This lethal effect was completely blocked by the addition of 25 μg of CM/ml to SM-sensitive cells 5 min before SM addition. Strain CSH-2-fi+ was resistant to CM and took up similar amounts of SM in the presence or absence of CM, providing further evidence that fi+ -containing cells are impermeable to CM.

Uptake of TC. All of the resistant and sensitive cells had an identical pattern of TC uptake during approximately the same for the sensitive and resistant cells.
50 min of incubation (Fig. 5). Since TC-resistant cells in the presence of TC had a demonstrated lag period before the onset of exponential growth, it seemed advisable to test for a permeability barrier before and after preincubation in TC. Resistant cells grown in the absence of TC were preincubated in TC for at least 2 hr, washed, and re-examined for uptake of radioactive TC. The resistant cells showed two different responses, depending upon the episome they carried. Those cells containing $fi^+$ episome were still as permeable to TC as nonpreincubated cells, whereas cells containing the $fi^-$ episome were relatively impermeable to TC (Fig. 5).

TC, which acts similarly to CM in that it is an inhibitor of protein synthesis and a bacteriostatic agent, was tested for its effect on SM action and uptake. As with CM, the uptake of SM by the sensitive cells was almost completely blocked by 25 $\mu$g of TC/ml (Fig. 6); TC also blocked the lethal effects of SM. The addition of 25 $\mu$g of TC/ml plus 5 $\mu$g of SM/ml to $fi^+$-containing cells not grown in TC resulted in only a 50% decrease of SM uptake. However, if the resistant cells were preincubated in the presence of TC for 2 hr before the addition of SM, the cells would then take up SM as well in the continued presence of TC as in its absence. TC apparently enters the cells initially and inhibits protein synthesis, but not sufficiently to block SM uptake entirely nor to prevent the biosynthesis necessary to set up the barrier to TC. After the adaptation period, the cells become firmly resistant to TC and no longer affect SM incorporation.

Effect of antibiotics on polyuridylic acid-dependent phenylalanine incorporation by cell-free extracts. Since antibiotics enter $fi^+$ cells, the mechanism of resistance may be due to an insensitive protein-synthesizing apparatus. Table 2 shows the results of several experiments testing the effects of SM and TC on the incorporation of phenylalanine into hot trichloroacetic acid-precipitable material with the use of extracts prepared from various resistant and sensitive cells.

SM and TC were both inhibitory when extracts were used from either sensitive or episomally resistant strains. This suggested that the mechanism of resistance in the resistant strains is not related to an insensitive protein-synthesizing
mechanism. The chromosomally resistant strain, W-677, on the other hand, had a protein-synthesizing system highly refractory to SM action.

Isoelectric point change. An attempt was made to demonstrate a difference in the isoelectric point of the cell surface of R+ or R- cells, by the method of acid agglutination. No differences could be detected; strains of E. coli K-12, 3000, and 180, with and without the R factor, gave isoelectric points of 3.70 ± 0.05. Neither the suppression of the F- antigen by the R factor nor the expression of drug resistance resulted in a change in the overall isoelectric point of the cells.

Antibiotic-inactivating enzymes. In some preliminary experiments, we tried to determine the presence in our resistant strains of an enzyme which might destroy TC. Neither whole cells nor cell-free extracts of TC-resistant cells showed any ability to decrease the inhibitory effects of TC on sensitive cells.

DISCUSSION

It is clear from various systems previously studied that drug resistance depends on the inability of a drug to reach its site of action in an active form, or else the site has been rendered immune to its action (7, 16).

The three drugs used in this study, TC, SM, and CM, all inhibit protein synthesis. Our data lead us to conclude that resistance to these drugs in cells containing either of the two types of R factors studied is not manifested by a drug-immune protein-synthesizing mechanism. A similar conclusion has been reached by others using fR-containing cells (17, 18). It has been found that some chromosomally drug-resistant strains do produce protein-synthesizing mechanisms relatively immune to drug action (1, 5, 24).

The two R factors differed, however, in determining permeability barriers to the drugs. Cells containing the fR factor appeared freely permeable to SM and TC, two drugs to which they are resistant. This observation, along with our inability to detect enzyme extracts active on the antibiotics and our studies indicating that the protein-synthesizing mechanism is sensitive to the antibiotics, does not allow us at this time to speculate on the mechanism of drug resistance in these strains. It is of interest to note that S. flexneri fR, which is resistant to 1,000 μg of SM/ml, is also freely permeable to SM, whereas an E. coli strain carrying the same episome is resistant only to 10 to 25 μg of SM/ml. The reason for this difference in resistance is not immediately apparent. The fR episome also determines resistance to CM. E. coli strains carrying this episome were relatively impermeable to CM, as compared with sensitive strains. This finding was supported by the observation that CM does not affect the uptake of SM in fR resistant strains as it does in sensitive cells, which was also observed by Hurwitz and Rosano (9).

The fR-containing cells were impermeable to TC and SM. Whereas the permeability barrier to SM was immediately evident, the onset of impermeability to TC required 2 hr of growth in the presence of TC. The resistant cells during this period apparently had the genetic determinant for resistance, but required a period of adaptation for the cells to become impermeable. Whatever the mechanism of resistance to TC in fR cells, there is also apparently this period of adaptation as noted by the lag required for their growth in the presence of this drug. In addition, it was noted in fR cells that, during this lag period, there was a partial inhibition of SM uptake in the presence of TC. This suggests that protein synthesis was partially inhibited during this period and that protein synthesis is required for the cell to resist SM uptake. In the fR cells, this protein synthesis is also apparently required for the synthesis of the permeability barrier to TC.
TABLE 2. Inhibition of polyuridylic acid-dependent phenylalanine incorporation by SM and TC in cell-free extracts of various strains of Escherichia coli K-12

<table>
<thead>
<tr>
<th>Antibiotic added to complete system</th>
<th>Conc of antibiotic</th>
<th>CSH-2-F&lt;sup&gt;+&lt;/sup&gt;</th>
<th>W-677</th>
<th>CSH-2-F&lt;sup&gt;-&lt;/sup&gt;</th>
<th>CSH-2-F&lt;sup&gt;+&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>Concentration (µg/ml)</td>
<td>Count/min&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Activity&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Count/min</td>
<td>Activity</td>
<td>Count/min</td>
</tr>
<tr>
<td>None</td>
<td>2,330</td>
<td>100</td>
<td>1,900</td>
<td>100</td>
<td>2,840</td>
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<tr>
<td>SM</td>
<td>0.1</td>
<td>2,300</td>
<td>99</td>
<td>1,980</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1,730</td>
<td>74</td>
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<td>1,430</td>
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<td></td>
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<td>100</td>
<td>960</td>
<td>48</td>
<td>1,150</td>
<td>55</td>
</tr>
</tbody>
</table>

<sup>a</sup> Complete system as previously described (14).
<sup>b</sup> The counts/minute of the complete system at zero-time has been subtracted from all values.
<sup>c</sup> Percentage of activity observed, compared with the activity of the complete system with no additions (set at 100%).

TABLE 3. Summary of results with sensitive and resistant cells<sup>a</sup>

<table>
<thead>
<tr>
<th>Cells used</th>
<th>SM</th>
<th>TC</th>
<th>CM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug uptake</td>
<td>Amino acid incorporation</td>
<td>Drug uptake</td>
<td>Amino acid incorporation</td>
</tr>
<tr>
<td>Sensitive</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>f&lt;sup&gt;+&lt;/sup&gt; (tet&lt;sup&gt;-&lt;/sup&gt; str&lt;sup&gt;-&lt;/sup&gt;r chl&lt;sup&gt;-&lt;/sup&gt;r)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>f&lt;sup&gt;-&lt;/sup&gt; (tet&lt;sup&gt;-&lt;/sup&gt; str&lt;sup&gt;-&lt;/sup&gt;r chl&lt;sup&gt;-&lt;/sup&gt;r)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chromosomal resistance&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> Symbols: + = freely permeable to drug, or amino acid incorporation inhibited in vitro; - = impermeable to drug, or insensitive to in vitro inhibition of amino acid incorporation.
<sup>b</sup> Cells containing the episome N<sub>2</sub> become impermeable only after 2 hr of incubation in the presence of TC.
<sup>c</sup> In this study, we examined only the chromosomal str<sup>-</sup>r cell; the other results are from published reports (6, 18, 24).

Impermeability can thus be postulated as a mechanism for resistance to TC and SM in f<sup>-</sup>-containing cells, and for CM resistance in f<sup>+</sup>-containing cells (Table 3). A well-known method of obtaining presumptive evidence for a permeability barrier is that of showing the availability of an enzyme or an enzyme system to an inhibitor only after cell rupture. By use of such a method, impermeability has been proposed as the mechanism of resistance to TC and SM in f<sup>-</sup> cells (17, 18). This observation is in contrast to the results of the present study, in which the direct method of drug uptake was used.

We have reported on our inability to find enzymes active on antibiotics in our episome-containing cells. Watanabe reported finding such enzymes active on TC and CM in episomally resistant cells (20). However, in those studies, the same amount of CM-specific enzyme was found in both sensitive and resistant cells, leading one to wonder about its role in resistance. Our inability to detect an enzyme active on TC leads us to question its importance in TC resistance in these strains, even if present in small quantities not detected by our assays.

We have observed that the same episome in two different strains leads to a great difference in the degree of resistance to SM. The determination of impermeability to TC and SM is dependent upon the type of R factor involved. Some chromosomally resistant strains produce protein-synthesizing mechanisms relatively immune to the drugs (1, 5, 24), whereas the resistant loci on the episomes do not. It is apparent that there are sev-
eral loci for resistance to each of the drugs, and that these loci determine different levels and different mechanisms of resistance. It appears also that a given gene can be expressed to different degrees, depending on the strain in which it is present.

ACKNOWLEDGMENTS

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LITERATURE CITED